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拟南芥中磷脂酶 Dδ 参与机械伤害诱导的磷脂酸生成*

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摘要: 磷脂酶水解磷脂产生磷脂酸 (phosphatidic acid, PA), Dα1 和 δ 是磷脂酶 D 家族中表达丰度最高的两个成员, 已知磷脂酶 Dα1 参与了机械伤害诱导的磷脂酸信号, 但是磷脂酶 Dδ 是否以及如何参与 PA 信号尚且未知。本研究利用脂类组学分析方法, 比较了拟南芥野生型 (WS) 和磷脂酶 Dδ 基因 T-DNA 插入突变体 (PLDδ-KO), 在机械伤害后的较长时间段 (6 h) 的膜脂分子变化。结果发现, 机械伤害后, 拟南芥两种基因型的大部分膜脂均发生下降, 且机械伤害后 30 min, PA 含量即快速并急剧升高; 随着时间的延长, 其水平持续升高, 直至达到峰值后下降至 6 h 达到最低值。WS 和 PLDδ-KO 达到 PA 最高值的时间不同, 分别为 1 h 和 3 h; 在伤害处理后 30 min 至 3 h 期间, PLDδ-KO 中的 PA 水平低于 WS, 两个基因型中的 PA 含量最大差值为 20%, 发生在伤害后 1 h。这证明缺失 *PLDδ* 基因在一定程度抑制了机械伤害诱导的 PA 生产, 表明 PLDδ 参与拟南芥响应机械伤害的 PA 生成, 但是其响应较 PLDα1 作用慢且轻。这是 PLDδ 响应拟南芥中机械伤害的首次报道。

关键词: 拟南芥; 机械伤害; 膜脂; 磷脂酶 Dδ; 磷脂酸
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Phospholipase Dδ is Involved in Wounding-Induced Phosphatidic Acid Formation in *Arabidopsis* *

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Abstract: Phosphalipase D (PLD) hydrolyzes phospholipids into phosphatidic acid (PA). PLDα1 and δ are the two most abundant members of the 12-member PLD family in *Arabidopsis*. PLDα1 has been demonstrated having role in the wounding-induced PA signalling. However, whether and how PLDδ is involved in wounding-induced PA formation remained unclear. In the present study, the membrane lipids response to wounding was profiled in Wassilewskija (WS) and PLDδ knockout mutant (PLDδ-KO) of *Arabidopsis*. The levels of most lipids, including monogalactosyldiacylglycerol, digalactosyldiacylglycerol, phosphatidylcholine and phosphatidylglycerol had decreased rapidly within 30 min after wounding in the two *Arabidopsis* genotypes. In contrast, the level of PA increased sharply and significantly 30 min after wounding. It continued to increase until peaking at 1 h post-wounding in WS and 3 h post-wounding in PLDδ-KO, and then decreased. The PA levels were similar in the two genotypes in untreated leaves and in leaves 6 h after wounding. However, these levels were lower in PLDδ-KO than in WS from 30 min to 3 h post-wounding. The significant difference of PA level between the two genotypes occurred 30 min after wounding, when it was about 20% lower in PLDδ-KO than in WS. These results show that PLDδ is involved in wounding-induced PA formation in *Arabidopsis*, but its absence induces PA response later and with less intensity than PLDα1.

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Key words: *Arabidopsis*; Wounding; Membrane lipid; PLDδ; Phosphatidic acid

Abbreviations: PLD, phospholipase D; DGDG, digalactosyldiacylglycerol; ESI, electrospray ionisation; MS/MS, tandem mass spectrometry; MGDG, monogalactosyldiacylglycerol; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine; ACL, acyl chain length; DBI, double-bond index

Herbivores constitute a threat to plants by inducing wounding and rapidly destroying plant tissues. Plants can also suffer damage from environmental stresses, such as wind, sand, hail and rain, which also produce mechanical wounding; this induces similar responses to those to wounding by herbivores (Buchanan *et al.*, 2004). In response to wounding, plants adopt both direct and indirect defensive strategies (Wasternack *et al.*, 2006). Wounding causes rapid activation of phospholipase D (PLD)-mediated hydrolysis as a result of a rapid accumulation of choline and phosphatidic acid (PA) (Ryu and Wang, 1996); the latter is used for the synthesis of jasmonic acid, a main responder to wounding (Wang *et al.*, 2000). The secondary messenger PA serves a wide range of signalling roles in plant responses to environmental stresses (Wang, 2004). PA induction by wounding has been observed in several plant species, including castor bean (Ryu and Wang, 1996), tomato, soybean, sunflower, broad bean, pepper (Lee *et al.*, 1997), tobacco (Dhondt *et al.*, 2000), and *Arabidopsis thaliana* (Ling *et al.*, 2007).

Multiple PLDs have been described in various plant species (Hanahan and Chaikoff, 1947; Qin *et al.*, 1997; Wang, 2000; Wang *et al.*, 1994; Xu *et al.*, 1997). The 12 PLD genes from *Arabidopsis* have been grouped into six classes—PLDα (3), β (2), γ (3), δ, ε and ζ (2)—based on their sequence similarities and biochemical properties (Zhang *et al.*, 2005). PLDα, PLDβ and PLDγ have been characterised in response to wounding in *Arabidopsis* (Pappan *et al.*, 1998; Pappan *et al.*, 1997a, b; Qin *et al.*, 1997; Wang, 1999). PLDα1 and PLDδ are the two most abundant members of the *Arabidopsis* PLD family. Wang *et al.* (2000) reported that the level of PA increased within 5 min after wounding, and the maximum difference in the level of PA between PLDα1-deficient and wild type leaves occurred 15 min after wounding, when that in the WT was 2.5-fold higher than that in the PLDα1-deficient strain (Wang *et al.*, 2000). PLDδ is involved in dehydration- and freezing- induced PA formation in *Arabidopsis* (Katagiri *et al.*, 2001; Li *et al.*, 2008). PLDα1 can compensate for the loss of PLDδ in PLDδ-KO mutant, and these two PLD isoforms together were shown to account for almost all the discernible activity seen in response to water deficit (Bargmann *et al.*, 2009). However, whether and if so, how PLDδ is involved in wounding-induced PA production remained unknown.

With the aim of determining the role of PLDδ in lipid metabolism in response to wounding, lipidomic profiling was carried out for *Arabidopsis* leaves in the Wassilewskija (WS) and PLDδ knockout (PLDδ-KO) genotypes, with and without wounding treatment. We used bioinformatics methods, including principal component analysis (PCA) and K-means/medians clustering, to analyse the function of PLDδ in wounding-induced lipid metabolism. Our results showed that about 20% of PA production upon wounding was attributable to PLDδ at the time of statistically significant difference in its level between WS and PLDδ-KO. Moreover, an absence of PLDδ slowed the PA response to wounding. The responses of desaturation and acyl chain length in membrane lipids to wounding were also discussed.

1 Materials and methods

1.1 Plant material, wound treatment and sampling

Leaves from approximately five-week-old *Arabidopsis* plants of WS and PLDδ-KO in a WS background were used for the identification and kinetic a-

nalysis of lipid species. The loss of *PLDδ* in the PLDδ-KO mutant of *Arabidopsis* has been proved by the absence of its transcript, protein and activity (Zhang *et al.*, 2003). The plants were cultivated in a walk-in chamber at 20−23 ℃, with a light intensity of 120 μmol $m^{-2}s^{-1}$, a 12-h photoperiod and 60% relative humidity. In addition, they were fully watered for five weeks until the beginning of the experiments. The method of wounding treatment was the same as that described by Ling *et al.* (2007).

1.2 Lipid extraction, ESI-MS/MS and data processing

Lipid extraction, sample analysis and data processing were performed as described previously with minor modifications (Li *et al.*, 2008; Welti *et al.*, 2002). Briefly, the leaves were harvested at the sampling time and transferred immediately into 2 mL of isopropanol with 0.01% butylated hydroxytoluene at 75 ℃. The tissue was extracted with chloroform/methanol (2∶1) three additional times with 2 h of agitation each time. The remaining plant tissue was heated overnight at 105 ℃ and weighed. Lipid samples were analysed using a triple quadrupole tandem mass spectrometry (MS/MS) equipped for electrospray ionisation (ESI). The lipids in each class were quantified by comparison with two internal standards for the class. Data processing was performed as previously described (Welti *et al.*, 2002). Five replicates from each sampling time were analysed. A Q-test was performed on the total amount of lipid in each head-group class, and data from discordant samples were removed (Welti *et al.*, 2002). The data were subjected to one-way analysis of variance (ANOVA) using SPSS 16.0. Statistical significance was tested by Fisher's least significant difference (LSD) method. The double-bond index (DBI) was calculated using the formula: DBI = (∑[N × mol% lipid])/100, where N is the number of double bonds in each lipid molecule (Rawyler *et al.*, 1999). Acyl chain length (ACL) was calculated using a formula derived from the DBI calculation above: ACL = (∑[NC × mol% lipid])/100, where NC is the number of carbon atoms in each lipid molecule.

The lipid contents of molecular species were used for assessing the differences among the samples (treatments and genotypes) by PCA with SPSS 16.0. More than 120 lipid contents of molecular species were transformed to a new set of variables, the principal components were ordered so that the first few retain most of the variation present in all of the original variables (Jolliffe, 2005).

2 Results

2.1 *A. thaliana* altered its lipid content and composition in response to wounding

The wounded leaves of WS and PLDδ-KO were sampled 0 and 30 min, and 1, 3 and 6 h after wounding. Lipids were profiled by a lipidomics approach based on ESI-MS/MS. We identified quantitatively more than 120 molecular species of glycerolipids, which included six head-group classes of phospholipids: phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylserine (PS), PA and phosphatidylglycerol (PG); as well as two head-group classes of galactolipids: monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG). Each molecular species was identified in terms of the total number of acyl carbon atoms and double bonds (Welti *et al.*, 2002). The total amount of lipid and the average level of molecular species in each head-group class are shown in Table 1 and Fig. 1.

The lipid content changed significantly in the leaves of *Arabidopsis* of both genotypes upon wounding treatment. The levels of DGDG, MGDG, PC and PI decreased significantly during the period after wounding. The extraplastidic lipids, PG, PC, PE and PI, degraded much more in response to wounding in PLDδ-KO than those in WS. It suggested that the absence of PLDδ might enhance the degradation of extraplastidic lipids in response to wounding. The lipid class that showed the largest change in absolute amount was PA, which increased sharply within 1 h after wounding treatment, and then gradu-

ally decreased in both genotypes. Its level was higher in WS than in PLDδ-KO *Arabidopsis* leaves at 30 min, 1 h and 3 h after wounding (Table 1). The level of PA rose sharply by nine- or thirteen-fold at 1 h in WS and at 3 h in PLDδ-KO after wounding, respectively (Table 1). Profiling more than 120 molecular species of membrane lipids in the leaves of both WS and PLDδ-KO genotypes, in terms of their absolute contents (Fig. 1), revealed that the levels of molecular species 34:6-MGDG and 36:6-DGDG, as well as all detected molecular species of PC, PG and PI, declined throughout the tested period after wounding. Meanwhile, levels of all PA molecular species increased in both genotypes. Given that 34:4-PG was the only molecular species detected with 34:4 acyls (Fig. 1), it was suggested that the increase in wound-induced 34:4-PA was derived from PLD-mediated conversion from PG. In fact, all of the molecular species of PA that increased might have been derived from PLD-mediated modification of PC, PE, PG, PI and PS (Wang, 2005). Therefore, PA molecular species appeared to reflect the rapid response of phospholipids to wounding damage in *Arabidopsis* leaves.

2.2 Principal component analysis revealed different responses of lipid molecular species to wounding

Differences among the samples (treatments and genotypes) were assessed using PCA with SPSS 16.0 The results of this analysis highlighted major statistically significant differences among the different periods after wounding. Analysis of the dataset allowed the extraction of nine principal components that explained 100% of the variance in the system (Table 2). The first three components, which are used for plotting the scores and loadings, explained 75% of the total variance (Table 2). Fig. 2 depicts two score plots.

Principal component 1 represents the lipid species of PA which are the class exhibited the largest changes and the lipid species of PC which are most abundant constitute of extraplastidic membrane lipid classes (Table 3); principal component 2 represents

Table 1　Amount of lipid in each head-group class in WS and PLDδ-KO *Arabidopsis* leaves at different times after wounding. The percentage of maximum relative change in lipids after wounding (Max RC) is the value for the maximum difference between the values of Control and different period after wounding, divided by the value of Control one. Values in the same row with different letters are significantly different ($P < 0.05$). Values are means ± standard deviation ($n = 4$ or 5)

Lipid class	Genotype	Lipid/dry weight (nmol/mg)					Max RC /%
		0	30 min	1 h	3 h	6 h	
DGDG	WS	35.98 ± 1.24^{a}	30.9 ± 2.92^{b}	30.42 ± 2.21^{bc}	27.52 ± 0.73^{c}	28.06 ± 2.09^{c}	−23.5
	PLDδ-KO	31.24 ± 1.14^{a}	28.3 ± 3.46^{ab}	26.62 ± 2.39^{b}	26.8 ± 4.44^{b}	28.77 ± 1.7^{ab}	−14.8
MGDG	WS	159.37 ± 3.53^{a}	133.41 ± 15.3^{b}	121.61 ± 28.39^{b}	121.42 ± 11.75^{b}	118.77 ± 8.71^{b}	−23.8
	PLDδ-KO	149.49 ± 10.3^{a}	125.47 ± 13.24^{b}	115.61 ± 11.48^{b}	118.1 ± 14.85^{b}	117.57 ± 11.41^{b}	−22.7
PG	WS	11.2 ± 1.44^{a}	10.51 ± 1.47^{a}	10.61 ± 0.71^{a}	9.98 ± 2.15^{a}	9.16 ± 1.64^{a}	—
	PLDδ-KO	11.5 ± 0.29^{a}	9.49 ± 1.1^{b}	7.7 ± 0.34^{c}	8.64 ± 0.98^{bc}	7.54 ± 1.87^{c}	−34.4
PC	WS	13.94 ± 1.62^{a}	12.79 ± 1.94^{ab}	12.03 ± 2.05^{ab}	11.12 ± 3.72^{b}	10.43 ± 0.8^{b}	−25.2
	PLDδ-KO	14.5 ± 1.72^{a}	12.19 ± 1.3^{b}	10.26 ± 0.14^{b}	10.57 ± 0.43^{b}	11.49 ± 1.25^{b}	−29.2
PE	WS	4.37 ± 1.05^{a}	5.11 ± 1.52^{a}	4.74 ± 1.21^{a}	4.62 ± 1.51^{a}	4.4 ± 0.64^{a}	—
	PLDδ-KO	5.53 ± 1.26^{a}	5.37 ± 0.97^{ab}	3.71 ± 0.44^{b}	4.13 ± 0.78^{ab}	4.57 ± 2.45^{ab}	−32.9
PI	WS	2.28 ± 0.28^{ab}	2.47 ± 0.12^{ab}	2.56 ± 0.48^{a}	2.24 ± 0.06^{ab}	2.17 ± 0.16^{b}	−4.8
	PLDδ-KO	2.35 ± 0.1^{a}	2.15 ± 0.31^{a}	1.72 ± 0.28^{b}	2.04 ± 0.27^{ab}	2.34 ± 0.23^{a}	−26.8
PS	WS	0.27 ± 0.06^{a}	0.33 ± 0.09^{a}	0.29 ± 0.06^{a}	0.29 ± 0.09^{a}	0.24 ± 0.03^{a}	—
	PLDδ-KO	0.29 ± 0.05^{ab}	0.33 ± 0.05^{a}	0.24 ± 0.05^{ab}	0.23 ± 0.04^{b}	0.25 ± 0.16^{ab}	—
PA	WS	0.4 ± 0.06^{c}	2.98 ± 0.36^{b}	4.17 ± 1.32^{a}	4.11 ± 1.21^{a}	2.68 ± 0.78^{b}	942.5
	PLDδ-KO	0.23 ± 0.2^{d}	2.31 ± 0.24^{c}	2.85 ± 0.06^{b}	3.31 ± 0.45^{a}	2.94 ± 0.35^{ab}	1339.1

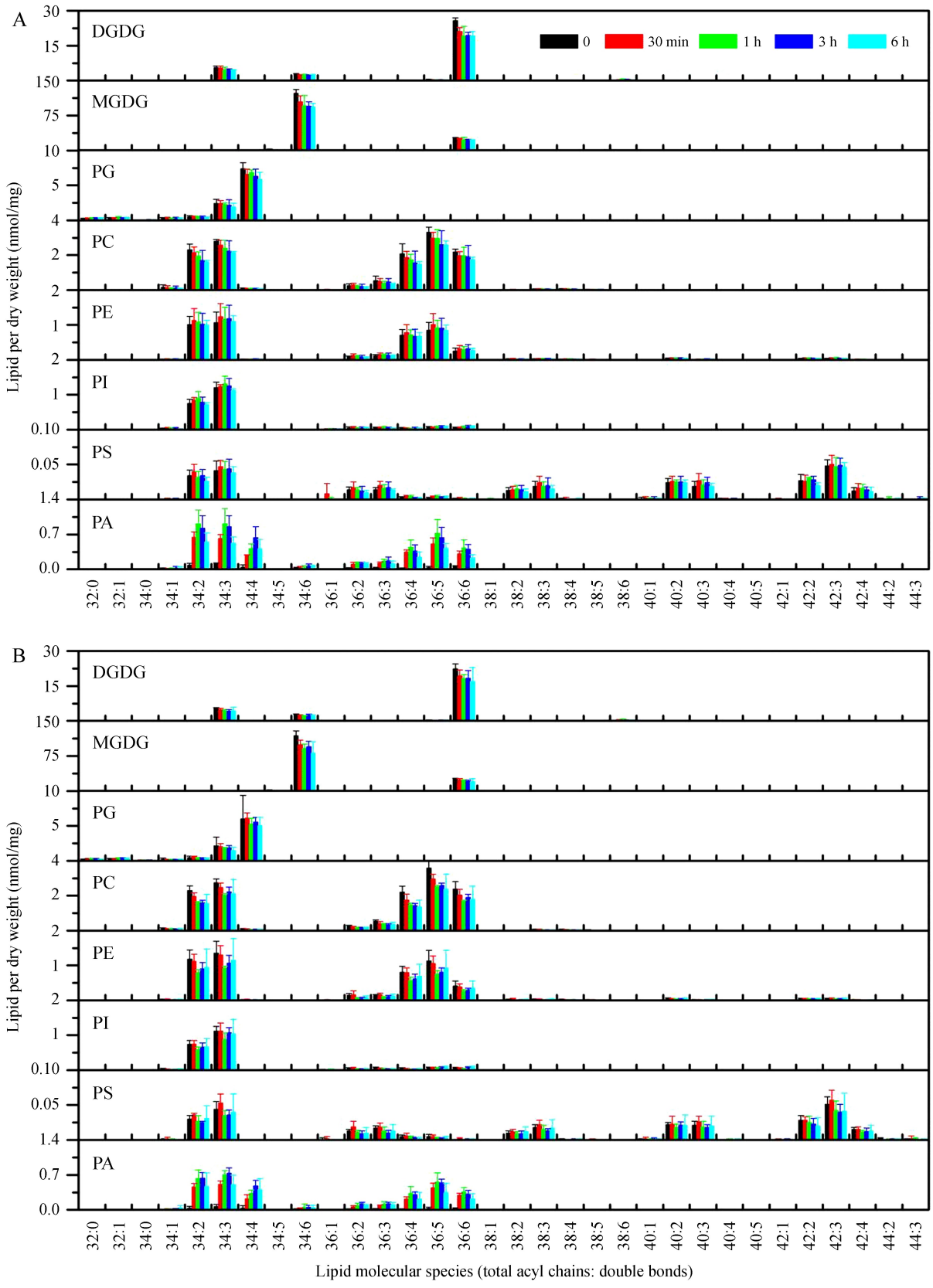

Fig. 1 Changes in the molecular species of membrane lipids in WS (A) and PLDδ-KO *Arabidopsis* (B), in terms of absolute contents. Values are means ± standard deviation (n=4 or 5)

Table 2　The nine principal components extracted by principal component analysis

Component	Extraction sums of squared loadings		
	Total	% of Variance	Cumulative %
1	51.837	42.144	42.144
2	24.702	20.083	62.227
3	15.789	12.836	75.064
4	8.884	7.223	82.286
5	6.721	5.464	87.750
6	5.155	4.191	91.942
7	4.237	3.445	95.387
8	3.517	2.859	98.246
9	2.158	1.754	100.000

the main plastidic component lipids, MGDG and DGDG (Fig. 2); principal component 3 basically represents lipids PE and PS, which differentiate the two genotypes (Fig. 2). The highest and lowest loading values (Table 3) are the lipid species that are most important in the assignment of each principal component. Examination of loadings (Table 3) clearly reveals that the separation of the points for the control treatment and for the timing of 30 min from those for 1, 3 and 6 h along the principal component 1 results primarily from the levels of PA and PC. There were lower levels of PA species and higher levels of PC species in fresh and 30 min post-wounding leaves than those in leaves of both genotypes from other periods post-wounding. This indicates that these are the most important lipid species for differentiation between the distinct periods after wounding. Principal component 3 clearly describes differences between the two genotypes, WS and PLDδ-KO (Fig. 2B). Examination of loadings (Table 3) reveals that the separation between WS and PLDδ-KO along the principal component 3 axis results primarily from the higher levels of PS and PE species in PLDδ-KO and the higher levels of 32:1- and 34:1-PG, and 34:2-DGDG in WS (Fig. 2), which indicates that these species are important in the statistical differentiation of WS from PLDδ-KO.

Plot of principal component 1 against principal component 2 showed the relationships and distances between the two genotypes at different times after wounding (Fig. 2A). The control of WS and PLDδ-KO cluster together, and the points for 30 min post-wounding of the two genotypes also cluster together. However, the points for WS and PLDδ-KO at 1 h and 3 h are scattered until at 6 h when they cluster together again (Fig. 2A). These patterns of separation and clustering basically occur along the principal component 1, which suggests that PA and PC mainly contribute these patterns of variation. Along principal component 3 (Fig. 2B), the differences between genotypes in same treatment were larger than between treatments in same genotype. This suggests that PE and PS do nothing with wounding, but contribute to the difference of two genotypes.

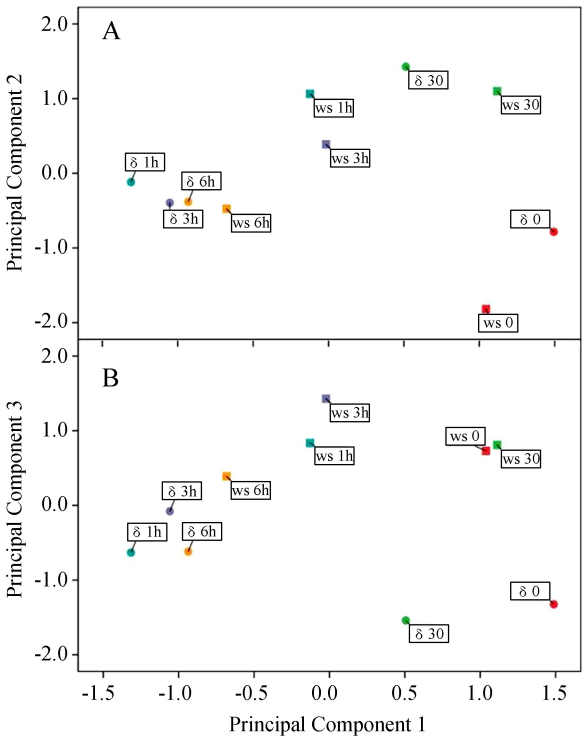

Fig. 2　Principal component analysis (PCA) of lipid molecular species in five time points post-wounding (treatments called here) among WS and PLDδ-KO *Arabidopsis*. The first three components, which accounted for 75% of the total data variance, were used for plotting the scores. Each point, a treatment and genotype combination is the mean of the corresponding replicates' principal component scores. (A) The score plot of principal component 1 (42% of the variance) vs. principal component 2 (20%). (B) The score plot of principal component 1 (42% of the variance) vs. principal component 3 (13%). WS, Wassilewskija; δ, PLDδ-KO; 0, control; 30', 30 minutes post-wounding; 1 h, 1 h post-wounding; 3 h, 3 h post-wounding; 6 h, 6 h post-wounding

Table 3 Loadings of the first three principal components. The twelve highest and twelve lowest loading values are indicated

Lipid species	Principal component 1 loadings	Lipid species	Principal component 2 loadings	Lipid species	Principal component 3 loadings
Twelve lowest loading values					
34:6 PA	−0.688	34:5 MGDG	−0.632	38:5 PS	−0.755
34:0 PG	−0.687	38:5 MGDG	−0.615	36:6 PE	−0.597
34:4 PA	−0.673	36:5 DGDG	−0.595	34:1 PS	−0.556
36:1 DGDG	−0.634	38:4 PS	−0.594	36:4 PS	−0.52
36:2 PA	−0.631	34:6 DGDG	−0.559	36:5 PS	−0.489
34:3 PA	−0.595	36:5 MGDG	−0.533	36:1 PE	−0.476
36:3 PA	−0.563	40:3 PC	−0.532	42:2 PE	−0.457
36:5 PA	−0.552	36:6 DGDG	−0.476	36:2 PE	−0.412
34:2 PA	−0.535	34:4 DGDG	−0.422	42:4 PE	−0.41
36:6 PA	−0.532	34:4 MGDG	−0.408	36:5 PE	−0.406
38:6 DGDG	−0.532	34:6 MGDG	−0.4	34:1 PE	−0.395
38:6 MGDG	−0.524	44:2 PS	−0.397	38:2 PE	−0.39
Twelve highest loading values					
36:5 PC	0.894	36:2 DGDG	0.69	34:6 PA	0.547
38:2 PC	0.895	36:4 PA	0.703	34:3 MGDG	0.565
34:5 DGDG	0.902	36:1 MGDG	0.719	34:1 MGDG	0.603
36:4 DGDG	0.904	36:5 PA	0.722	34:4 PI	0.609
34:4 PC	0.909	36:6 PA	0.726	32:0 PG	0.613
36:3 PC	0.914	42:3 PE	0.739	38:2 PS	0.631
36:6 MGDG	0.916	38:6 MGDG	0.747	34:3 PI	0.633
34:3 DGDG	0.931	40:3 PS	0.747	40:2 PS	0.64
36:4 PC	0.933	38:3 MGDG	0.76	36:6 PI	0.651
36:2 PC	0.943	36:2 MGDG	0.766	34:1 PG	0.726
34:3 PC	0.943	38:4 MGDG	0.771	32:1 PG	0.762
34:2 PC	0.947	38:6 PE	0.848	34:2 MGDG	0.799

2.3 PA transiently increases and reacts differently to wounding in the WS and PLDδ-KO *Arabidopsis*

The PCA result suggested that PA was one of the main principal component factors involved in the response to wounding in WS and PLDδ-KO *Arabidopsis*. Therefore, we further used expression pattern analysis with Mev 4.9.0 to analyse in details the variation of PA species after wounding in the two genotypes.

The results show that the PA level transiently increased upon wounding in the both genotypes (Fig. 3A−K). the levels of PA and several of its main species in control leaves in PLDδ-KO were similar to those of WS (Fig. 3A−I). This suggests that an absence of PLDδ does not influence the level of PA in control leaves in *Arabidopsis*. At 30 min after wounding, the content of PA and several of its main species increased sharply; particularly large increases were found for 36:4- and 36:5-PA, which increased 30- and 17-fold in PLDδ-KO, and 27- and 17-fold in WS, respectively (Fig. 1A, B); subsequently, most of them continued to increase to the highest level at 1 h after wounding, and then decreased until 6 h after wounding (Fig. 3A−I). However, there were still several differences of PA variation in response to wounding between the two genotypes. Firstly, the levels of PA and nearly all of its species in PLDδ-KO were lower than those in WS (Fig. 3A−D, F−I) from 30 min to 3 h after wounding. In WS, the PA level increased to the highest level of 4.17 nmol · mg^{-1}, but that in PLDδ-KO was 3.31 nmol · mg^{-1}, this suggests that suppression of *PLDδ* caused a 20% decrease of the PA level in *Arabidopsis*. Secondly, the peak PA levels during the post-wounding period differed: namely, the peak occurred at 1 h in WS, but at 3 h in PLDδ-KO

(Fig. 3J, K). This suggested that a lack of PLDδ slows the early response of PA. Finally, the difference of PA levels between the two genotypes to wounding decreased over time. This suggested that PLDδ had a rapid response to wounding.

2.4 The responses of unsaturation and acyl chain length to wounding in WS and PLDδ-KO *Arabidopsis*

The level of unsaturation level (Quartacci *et al.*, 2002) and acyl chain length (Denich *et al.*, 2003) of phospholipids and glycolipids in membranes could affect membrane fluidity . The DBI was calculated by the double bond of lipids and ACLs was calculated by the total number of acyl carbon atoms of lipids. We analysed the DBI and ACL of lipid classes in response to wounding in *Arabidopsis* (WS and PLDδ-KO). After wounding, the DBI of plastidic membrane lipids PG decreased significantly in both

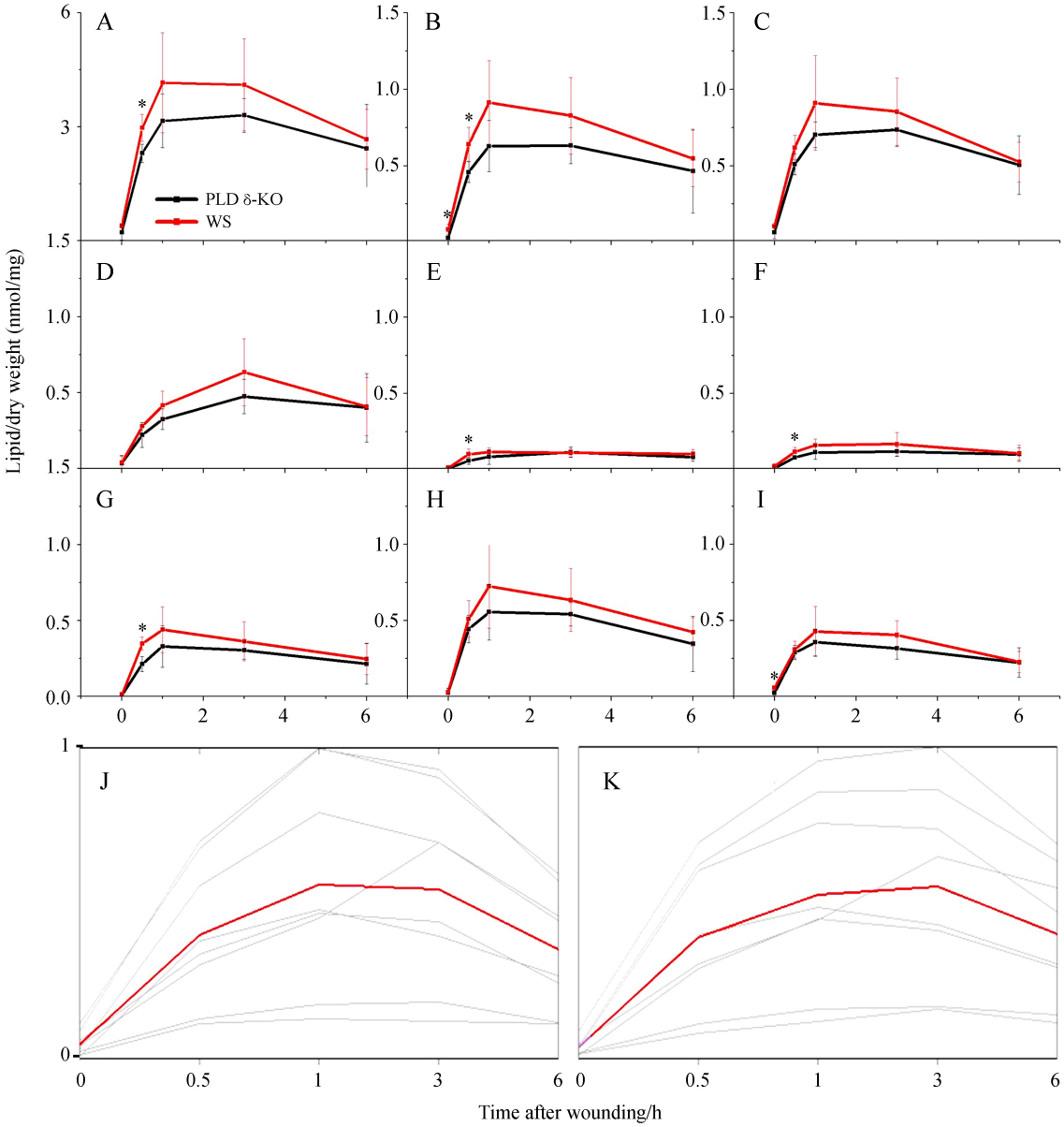

Fig. 3　Changes of the levels of PA and its molecular species after wounding in WS and PLDδ-KO *Arabidopsis* leaves. A–I. Detected levels of PA and its species (A, PA; B, 34∶2-PA; C, 34∶3-PA; D, 34∶4-PA; E, 36∶2-PA; F, 36∶3-PA; G, 36∶4-PA; H, 36∶5-PA; I, 36∶6-PA). J–K. The curve upon combining eight PA species in WS (J) and PLDδ-KO (K). "＊" indicates that the value is significantly different from that for WS under the same condition ($P < 0.05$). Values are means ± standard deviation ($n = 4$ or 5)

genotypes, whereas those of extra-plastidic membrane lipids—PE, PI, PS and PA— almost increased (Table 4). The maximum changing of DBI after wounding was that of PG, which decreased 0.08, and 0.11 in WS and PLDδ-KO separately. The ACL of most lipid classes remained unchanged after wounding. However, that of the plastidic lipid (including MGDG, DGDG, and PG) changed significantly after wounding (Table 5). Basically, DBI of lipid classes respond to wounding, but only ACL of plastidic lipids respond to wounding in *Arabidopsis* (both WS and PLDδ-KO).

3 Discussion

After the profiling and analysis of lipids for a relatively long period of 6 h after wounding in WS and PLDδ-KO *Arabidopsis*, we found that levels of the majority of lipids were reduced rapidly in both WS and PLDδ-KO leaves after wounding. However, only level of PA increased significantly in response to wounding. PLDδ also influenced the degradation of some lipids, such as lipids PE, PI and PG, which decreased their amounts sharply in PLDδ-KO leaves, but were unchanged at the same time points in WS after wounding for later than 1 h. PLDδ partly regulates PA levels induced by wounding. During the whole period after wounding, the most dramatic changes of lipids occurred 30 min after wounding. Thirty minutes is a limit time, but appears to be sufficient for lipids in *Arabidopsis* to respond to wound damage, especially in the case of the lipid PA. This rapid response suggests that PA might act as a signalling molecule in response to wounding. The rapid lipid hydrolysis was the same as that reported previously in wounded tissues of various plants (Lee *et al.*, 1997; Ling *et al.*, 2007; Lojkowska, 1988; Zien *et al.*, 2001). However, the trend of variation of different lipid classes at the same time points differed; for example, in our work on *Arabidopsis*, lipid PE did not change among the time points after wounding in WS, but it decreased significantly reported in Zien *et al.* (2001).

Table 4 DBI values of membrane lipids in WS and PLDδ-KO *Arabidopsis* leaves at different times after wounding. The values of maximum relative change (Max RC) is the maximum values for the difference between the DBI values after wounding and control. Values in the same row with different letters are significantly different different ($P < 0.05$).

Values are means ± standard deviation ($n = 4$ or 5)

Lipid class	Genotype	Double Bond Index (DBI)					Max RC /%
		0	30 min	1 h	3 h	6 h	
DGDG	WS	5.43 ± 0.07^{a}	5.35 ± 0.04^{b}	5.39 ± 0.05^{a}	5.38 ± 0.04^{a}	5.39 ± 0.04^{a}	−0.08
	PLDδ-KO	5.37 ± 0.02^{a}	5.37 ± 0.08^{a}	5.4 ± 0.01^{a}	5.4 ± 0.04^{a}	5.34 ± 0.04^{a}	—
MGDG	WS	5.94 ± 0.02^{a}	5.95 ± 0.01^{a}	5.95 ± 0.01^{a}	5.94 ± 0.01^{a}	5.95 ± 0^{a}	—
	PLDδ-KO	5.95 ± 0.01^{b}	5.96 ± 0.01^{a}	5.96 ± 0.01^{a}	5.95 ± 0.01^{ab}	5.94 ± 0.01^{b}	0.01
PG	WS	3.43 ± 0.06^{a}	3.37 ± 0.01^{a}	3.36 ± 0.05^{a}	3.35 ± 0.06^{b}	3.35 ± 0.07^{b}	−0.08
	PLDδ-KO	3.45 ± 0.04^{a}	3.38 ± 0.06^{b}	3.34 ± 0.02^{b}	3.35 ± 0.07^{b}	3.41 ± 0.04^{ab}	−0.11
PC	WS	2.99 ± 0.04^{a}	2.97 ± 0.02^{a}	3 ± 0.06^{a}	3.02 ± 0.04^{a}	3.03 ± 0.02^{a}	—
	PLDδ-KO	3.03 ± 0.06^{a}	3.03 ± 0.02^{a}	3.02 ± 0.02^{a}	3.06 ± 0.08^{a}	3.02 ± 0.07^{a}	—
PE	WS	3.46 ± 0.01^{b}	3.48 ± 0.03^{ab}	3.48 ± 0.02^{ab}	3.5 ± 0.01^{a}	3.48 ± 0.03^{ab}	0.04
	PLDδ-KO	3.52 ± 0.07^{a}	3.51 ± 0.05^{a}	3.53 ± 0.03^{a}	3.53 ± 0.03^{a}	3.55 ± 0.05^{a}	—
PI	WS	2.78 ± 0.02^{b}	2.74 ± 0.03^{b}	2.76 ± 0.03^{b}	2.83 ± 0.02^{a}	2.82 ± 0.04^{a}	0.05
	PLDδ-KO	2.77 ± 0.03^{b}	2.76 ± 0.04^{b}	2.76 ± 0.02^{b}	2.84 ± 0.05^{a}	2.81 ± 0.07^{ab}	0.07
PS	WS	2.67 ± 0.02^{b}	2.68 ± 0.05^{b}	2.68 ± 0.05^{b}	2.66 ± 0.02^{b}	2.73 ± 0.02^{a}	0.06
	PLDδ-KO	2.71 ± 0.05^{b}	2.76 ± 0.03^{a}	2.7 ± 0.02^{b}	2.71 ± 0.04^{b}	2.72 ± 0.02^{ab}	0.05
PA	WS	3.56 ± 0.38^{a}	3.64 ± 0.05^{a}	3.63 ± 0.15^{a}	3.67 ± 0.05^{a}	3.63 ± 0.06^{a}	—
	PLDδ-KO	3.64 ± 0.08^{b}	3.75 ± 0.06^{a}	3.65 ± 0.05^{ab}	3.66 ± 0.08^{ab}	3.58 ± 0.12^{b}	0.11

Table 5　ACL values of membrane lipids in WS and PLDδ-KO *Arabidopsis* leaves at different times after wounding. The values of maximum relative change (Max RC) is the maximum values for the difference between the ACL values after wounding and control. Values in the same row with different letters are significantly different ($P < 0.05$).
Values are means ± standard deviation ($n = 4$ or 5)

Lipid class	Genotype	Acyl chain length					Max RC /%
		0	30 min	1 h	3 h	6 h	
DGDG	WS	35.51 ± 0.04^{a}	35.48 ± 0.03^{b}	35.51 ± 0.04^{a}	35.49 ± 0.02^{a}	35.49 ± 0.03^{a}	−0.03
	PLDδ-KO	35.46 ± 0.02^{a}	35.46 ± 0.02^{a}	35.5 ± 0.04^{a}	35.48 ± 0.04^{a}	35.46 ± 0.03^{a}	—
MGDG	WS	34.37 ± 0.01^{b}	34.4 ± 0.02^{a}	34.4 ± 0.02^{a}	34.39 ± 0.03^{ab}	34.39 ± 0.02^{ab}	0.03
	PLDδ-KO	34.37 ± 0.03^{a}	34.4 ± 0.02^{a}	34.4 ± 0.03^{a}	34.37 ± 0.03^{a}	34.39 ± 0.05^{a}	
PG	WS	33.89 ± 0.01^{a}	33.87 ± 0.01^{b}	33.86 ± 0.02^{b}	33.86 ± 0.01^{b}	33.85 ± 0.01^{b}	−0.04
	PLDδ-KO	33.92 ± 0^{a}	33.87 ± 0.01^{b}	33.87 ± 0^{b}	33.86 ± 0.02^{b}	33.89 ± 0.04^{ab}	−0.06
PC	WS	35.29 ± 0.05^{a}	35.29 ± 0.03^{a}	35.29 ± 0.04^{a}	35.3 ± 0.04^{a}	35.3 ± 0.02^{a}	—
	PLDδ-KO	35.33 ± 0.03^{a}	35.28 ± 0.02^{b}	35.31 ± 0.03^{ab}	35.3 ± 0.06^{ab}	35.29 ± 0.04^{ab}	0.05
PE	WS	35.25 ± 0.05^{a}	35.29 ± 0.02^{a}	35.29 ± 0.0^{a}	35.25 ± 0.04^{a}	35.26 ± 0.04^{a}	—
	PLDδ-KO	35.29 ± 0.02^{a}	35.33 ± 0.06^{a}	35.3 ± 0.04^{a}	35.28 ± 0.05^{a}	35.33 ± 0.07^{a}	—
PI	WS	34.25 ± 0.06^{a}	34.28 ± 0.02^{a}	34.24 ± 0.04^{a}	34.29 ± 0.04^{a}	34.28 ± 0.05^{a}	—
	PLDδ-KO	34.3 ± 0.03^{a}	34.24 ± 0.08^{a}	34.27 ± 0.03^{a}	34.28 ± 0.03^{a}	34.28 ± 0.02^{a}	—
PS	WS	38.29 ± 0.3^{a}	38.08 ± 0.2^{a}	38.38 ± 0.21^{a}	38.34 ± 0.25^{a}	38.36 ± 0.44^{a}	—
	PLDδ-KO	38.32 ± 0.16^{a}	38.13 ± 0.35^{a}	38.39 ± 0.47^{a}	38.4 ± 0.35^{a}	37.76 ± 1.1^{a}	—
PA	WS	34.76 ± 0.12^{a}	34.93 ± 0.04^{a}	34.89 ± 0.06^{a}	34.82 ± 0.04^{a}	34.83 ± 0.07^{a}	—
	PLDδ-KO	34.76 ± 0.23^{a}	34.94 ± 0.11^{a}	34.91 ± 0.08^{a}	34.85 ± 0.03^{a}	34.82 ± 0.07^{a}	—

Among all the tested lipid classes, PA exhibited the greatest increase in response to wounding. Specifically, its level varied after wounding, and the patterns of variation were basically similar between the two genotypes: a trend of a rise followed by a fall. PLDα1 was another main member of the PLD family that responded to wounding (Wang *et al.*, 2000). The level of PA still increased rapidly in PLDδ-KO, which suggested that the lack of PLDδ did not influence the reaction of PLDα1 to wounding. The function of PLDδ mainly exhibited during the period of 30 min to 3 h after wounding (Fig. 3A, Table 1). It decreased the level of PA by about 20% at 30 min after wounding in *Arabidopsis* (Fig. 3A, Table 1), which suggests that about 20% of PA production at this time point in response to wounding is attributable to PLDδ. Meanwhile, Wang *et al.* (2000) mentioned about 60% of wound-induced PA was attributable to PLDα1, another member of the PLD family, in *Arabidopsis*; in that case, PA responded to wounding five minutes after wounding treatment. In summary, PLDδ has a role in wounding-induced PA formation in *Arabidopsis*, but it reacts later and with less intensity than PLDα1.

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